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Influence of Water Stress on Nitrogen Fixation in Cowpea

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Abstract. Two greenhouse studies were conducted to investigate the relationship between water stress and N₂ fixation among drought-resistant and susceptible cowpea [*Vigna unguiculata* (L.) Walp.] genotypes. In both experiments, seeds were planted in 7.6-liter black polyethylene pots containing composted sawdust medium and were inoculated with *Rhizobium*. Throughout the experiments, flowers were removed to maintain vegetative growth. Water stress treatments were imposed by withholding water, while the control plants were watered as needed. The treatments were applied 58 and 56 days after planting (DAP) in the first and 2nd experiments, respectively. In both experiments, leaf water potential (LWP), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), nodule fresh weight (NW), nodule number (NN), and plant specific activity (PSA) by both in situ and destructive acetylene reduction methods were measured. Repeated observations of in situ acetylene reduction were made 58, 63, and 71 DAP in the first experiment. All other variables were measured 77 to 78 DAP in the first experiment. Single observations of all variables, including in situ and destructive acetylene reduction were made 56, 67, and 81 DAP in the 2nd experiment. Results suggested that resistant genotypes are capable of maintaining LWP and biomass production (as measured by SDW and SFW) during water stress. In addition, the effect of water stress on N₂ fixation was far greater than the influence of genotype when genotypes were selected for relative drought resistance. Path analysis revealed that LWP is correlated to N₂ fixation in water-stressed plants, and improvement of plant water status via drought resistance should increase N₂ fixation potential under drought conditions. Therefore, breeding for drought resistance in conjunction with N₂ fixation may be more beneficial than breeding strictly for N₂ fixation potential without regard for environmental adaptation. The in situ method of acetylene reduction was found to be useful for detecting physiological changes due to water stress and estimating its genotypic N₂ fixation potential.

Nodulation and nitrogenase activity in cowpea are reduced by drought-induced water stress, resulting in low N₂ fixation as well as reduced yields (2, 4, 9, 18, 22). In these experiments, the effect of water stress on nodulation and nitrogenase activity of only one cultivar was reported (4, 22), or when several cultivars were used, they were of unknown drought resistance (2, 18). Nitrogen fixation is considered to be more sensitive to water stress than to N uptake and assimilation of combined N (8). The possibility of selecting cowpea rhizobial strains for their capacity to recover from drought stress has been investigated (21). However, since drought affects many plant processes in addition to N₂ fixation, drought resistance of the cowpea host plant also should be beneficial. The effect of water stress on plant growth and N₂ fixation must be assessed to determine whether it is possible to increase N₂ fixation via drought resistance.

Traditional methods of measuring nitrogenase activity have involved destruction of the plant, which makes repeated observations impossible. For certain experiments, it may be desirable to measure environmental effects that elicit physiological changes over a period of time. Therefore, a nondestructive in situ method for measuring acetylene reduction was used similar to that reported by Mahon and Salminen (6).

Turk and Hall (10) separated drought resistance into 2 com-

ponents: drought avoidance and drought tolerance. They defined drought avoidance as the maintenance of high plant water potentials during drought (3, 10) and drought tolerance as the maintenance of plant function even when plant water potentials are low due to drought stress (10). Drought avoidance in cowpeas has been well documented (3, 10, 18). The effect of drought avoidance on N₂ fixation, however, has not been well-defined.

The objective of the studies reported herein was to determine the influence of water stress on the N₂-fixation potential of drought resistant and susceptible genotypes in order to assess the value of drought resistance as a means of increasing N₂ fixation potential in cowpea.

Materials and Methods

Two greenhouse experiments were conducted to determine the effect of water stress on N₂ fixation. In the first experiment, 14 genotypes, including 6 drought-resistant and 3 drought-susceptible (12-16), were planted on 21 Sept. 1981. The relative drought-resistance potential of the resistant and susceptible genotypes had been determined previously on the basis of shoot biomass production in field experiments conducted during Summer 1980 (14). The genotypes used in this study were selected on the basis of their resistance or susceptibility, as well as seed availability, and do not necessarily represent the extremes observed.

Five check cultivars were grown for comparison to exotic genotypes. 'Brown Crowder' and 'Bush Purple Hull' are high and low N₂-fixing genotypes, respectively (23). TX 101 and TX 351 are selections from 'Bush Purple Hull' and 'Brown Crowder', respectively. 'California Blackeye No. 5' has been used extensively in water stress research (7, 10, 11, 22). Plants were grown in 7.6-liter black plastic pots in composted sawdust, which has a low N content and is very porous. Seeds were inoculated at planting with the commercial mixed strain "EL"

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rhizobial inoculant. Seedlings were thinned later to one plant per pot. Plants were watered with a modified N-free nutrient solution (Table 1). Flowers were removed daily to reduce confounding among exotic lines differing in reproductive capacity and daylength sensitivity. Gro-Lux wide spectrum lighting was used to extend the daylength to about 16 hr.

All plants were watered with nutrient solution as needed for the first 58 days after planting (DAP). Plants assigned to the stress treatment were not watered beyond 58 DAP. Plants used as controls were watered with tap water 2 days prior to each in situ acetylene reduction measurement. Treatments were applied in a factorial arrangement in a randomized complete block design with 4 blocks. At 58, 63, and 71 DAP, pots were weighed to monitor the depletion of water from the medium, and in situ acetylene reduction assay was conducted between 1100 and 1300 HR on warm, sunny days (6). Septum-fitted glass tubing (15 cm long) was inserted into the sawdust medium parallel to the stem at a distance of 5 cm, and acetylene (50 cc) was injected. Gas samples obtained prior to acetylene injection had no detectable levels of ethylene. Gas was sampled at 10 and 60 min after acetylene injection for later ethylene determination. Plant specific activity, as determined by in situ acetylene reduction, was analyzed as a repeated-observation, split-plot design with sampling dates as main plots, genotypes and treatments as sub-plots, and sampling times as sub-sub-plots.

Leaf water potential (LWP) was determined 77 DAP by thermocouple psychrometry. At about 1300 HR, a 40-mm square leaf sample was obtained from the middle leaflet of the youngest, fully expanded trifoliate of each plant. Leaf samples were inserted rapidly into a Wescor L-44 waterbath leaf chamber. Leaf water potential was determined with a Wescor HR-33T dewpoint microvoltmeter (17).

The next day, plants were assayed (between 1100 and 1300 HR) for nitrogenase activity by the destructive acetylene reduction method. Plants were severed at the medium line, and shoots were weighed immediately and dried for later dry weight mea-

surement. The intact root system, including nodules and roots, was placed in an air-tight canning jar (0.48 liter). The acetylene reduction assay was used as described previously (23), with 50 cc of acetylene injected into the jar. After the assay, each root was placed in a plastic bag and frozen for later nodule weight (NW), nodule number (NN), and root fresh weight (RFW) measurements.

Most data were analyzed by analysis of variance (ANOVA). Whenever appropriate, single degree of freedom contrasts were used to detect differences in plant growth variables between resistant and susceptible genotypes. The relationships among various N₂-fixation variables were determined by path analysis (5).

A similar study was conducted during Spring 1982. The primary difference was that all variables, including both in situ and destructive acetylene reduction methods, were measured on 3 days—56, 67, and 81 DAP.

On each of the 3 harvest days, PSA of one-third of the plants was determined first in situ, followed by the destructive method; hence, one observation per plant was obtained with each method. Two drought-resistant and 2 susceptible genotypes, in addition to 'California Blackeye No. 5', were planted on 29 Jan. 1982 in a randomized complete block, split-split plot design with 5 blocks. Main plots were the harvest dates, subplots were the stress treatments, and sub-sub-plots were genotypes. In situ acetylene reduction was analyzed as a split-split-split-plot design with sampling times, 10 and 30 min after acetylene injection, as sub-sub-sub-plots. For this experiment, the amount of sawdust media used in each pot was weighed carefully. Plants were grown as before with the stress treatments applied 56 DAP.

Results and Discussion

Significant differences between the 2 stress treatments were detected for SFW, SDW, RFW, LWP, PSA, NW, and NN on 1 and 2 Dec. 1981 (Table 2). The stress treatment reduced shoot fresh and dry weight by 56% and 45%, respectively. Water stress reduced RFW and LWP by 32% and 49%, respectively, and PSA, NW, and NN by 61%, 44%, and 25%, respectively. These results indicated that the stress treatment had a significant and negative effect on plant growth and N₂ fixation.

Significant genotypic differences ($P < 0.05$) were detected for LWP, RFW, NW, NN, and in situ and destructive PSA. Single degree of freedom contrasts revealed significant differences between resistant and susceptible genotypes for only LWP (Table 3). Resistant genotypes generally had higher LWP than susceptible genotypes, although several of the check cultivars also had relatively high LWP (Table 3). Drought avoidance in cowpea has been reported previously (1, 3, 7, 10, 11, 18),

Table 1. Composition of N-free nutrient solution.

Stock solution	Stock solution concentration (g/100 ml)	Stock solution added to 100 liters H ₂ (ml)	Final concentration (mg element/liter)
Solution 1			
H ₃ BO ₃	0.3		(B) 0.0524
MnCl ₂	0.2		(Mn) 0.0873
ZnCl ₂	0.01		(Zn) 0.0048
CuCl ₂ ·2H ₂ O	0.005		(Cu) 0.0019
Na ₂ MoO ₄ ·2H ₂ O	0.0025	10	(Mo) 0.0010
Solution 2			
MgSO ₄	12.0	100	(Mg) 24.2400 (S) 31.9560
Solution 3			
KH ₂ PO ₄	13.0	100	(K) 37.3490 (P) 29.5880
Solution 4			
CaCl ₂	11.0	50	(Ca) 19.8610
Solution 5 ^a			
K ₂ SO ₄	13.0	100	(K) 58.3440 (S) 41.6780
Solution 6			
Fe-EDTA	5.0	10	(Fe) 0.5000

^aAdjusted to pH 6.5 with H₂SO₄.

Table 2. The influence of water stress on plant growth and N₂ fixation averaged over all genotypes, Fall 1981.

Variable	Treatment		Significance levels	SE
	Control	Stressed		
Shoot fresh weight (g)	32.0	14.0	***	1.81
Shoot dry weight (g)	5.5	3.0	***	0.35
Root fresh weight (g)	5.0	3.4	***	0.82
Leaf water potential (bars)	-9.6	-14.6	***	0.35
Plant specific activity (μmol C ₂ H ₄ ·plant ⁻¹ ·hr ⁻¹)	3.6	1.4	***	1.44
Nodule fresh weight (mg/plant)	1294	724	**	88.46
Nodule number/plant	115	86	**	2.36

****Significant at 1% and 0.1% levels, respectively.

Table 3. The influence of genotype on leaf water potential, Fall 1981.

Genotype ^a	Leaf water potential (bars)
Resistant^b	
TVu 129	-10.79
TVu 966	-11.68
TVu 1489	-11.85
TVu 2157	-13.72
TVu 2319	-11.95
TVu 4534	-11.53
Mean	-11.95
Susceptible	
Purple Tip	-14.60
TVu 6441	-13.17
TVu 6565	-12.72
Mean	-13.50
Check	
Brown Crowder	-13.37
Bush Purple Hull	-11.63
California Blackeye No. 5	-10.40
TX 101 selection	-10.82
TX 351 selection	-12.28
Mean	-11.70
SE	0.82

^aTVu members as cited in Cowpea Germplasm Catalog, No. 1, 1974, International Institute of Tropical Agriculture, Ibadan, Nigeria.

^bA contrast of resistant vs. susceptible genotypes was significant at the 0.05 level for leaf water potential.

*SE = Standard error of a difference between 2 means.

although differences between cowpea lines for drought avoidance have not been well-documented. The increased LWP values for check cultivars indicated that named cultivars possessed at least some drought avoidance traits. 'California Blackeye No. 5' was the most notable example. Nevertheless, exotic genotypes identified as resistant in this experiment and in previous field experiments (14) may express specific drought avoidance traits not present in commonly used named cultivars. Specific drought adaptation mechanisms in drought-resistant, exotic cowpea genotypes should be identified.

Since in situ PSA was not measured in a sealed container as with the destructive acetylene reduction assay, it cannot be assumed that acetylene reduction measured at 10 or 60 min represents a cumulative total. In fact, in situ PSA was 0.37 and 0.29 $\mu\text{mol C}_2\text{H}_4\cdot\text{plant}^{-1}$, 10 and 60 min after injecting acetylene, respectively. In situ PSA is, therefore, expressed as $\mu\text{mol C}_2\text{H}_4\cdot\text{plant}^{-1}$ without a unit for time.

The interaction between stress treatment and genotype was found to be significant for only PSA measured by the in situ method in the first experiment (Table 4). The largest differences among genotypes were in the control treatment, with drought-resistant genotypes, as a group, having lower in situ PSA than either susceptible or check genotypes. In the stress treatment, differences among genotypes were small, with all 3 groups having similar levels of nitrogenase activity as measured by in situ acetylene reduction. The most obvious differences between resistant, susceptible, and check genotypes were noted when in situ PSA in the stress treatment was expressed as a percentage of reduction (Table 4). Resistant genotypes were not affected as severely by water stress as were susceptible genotypes. Certain resistant genotypes (TVu 129, TVu 1489, and TVu 2319) were low N_2 fixers in the control treatment, but their reduction in N_2 fixation due to water stress was minimal. These 3 geno-

Table 4. The influence of genotype and stress treatment on plant specific activity as measured by the in situ acetylene reduction method, Fall 1981.

Genotype ^a	Plant specific activity ^c		Reduction (%)
	Stress treatments		
	Control	Stressed	
Resistant			
TVu 129	0.19 ^b	0.18	5
TVu 966	0.52	0.20	62
TVu 1489	0.23	0.19	17
TVu 2157	0.34	0.18	47
TVu 2319	0.28	0.234	14
TVu 4534	0.46	0.16	65
Mean	0.34	0.19	44
Susceptible			
Purple Tip	0.51	0.15	71
TVu 6441	0.52	0.22	58
TVu 6565	0.73	0.58	
Mean	0.59	0.20	66
Check			
Brown Crowder	0.56	0.20	64
Bush Purple Hull	0.54	0.14	74
California Blackeye No. 5	0.65	0.23	65
TX 101 selection	0.48	0.22	54
TX 351 selection	0.54	0.23	57
Mean	0.55	0.20	64
SE ^b	0.06		

^cPlant specific activity expressed as $\mu\text{mol C}_2\text{H}_4\cdot\text{plant}^{-1}$.

^aTVu numbers as cited in Cowpea Germplasm Catalog, No. 1, 1974, International Institute of Tropical Agriculture, Ibadan, Nigeria.

^cThe interaction between genotype and stress treatment was significant at the 0.05 level.

*SE = Standard error of a difference between 2 means.

types demonstrated some degree of drought avoidance by maintaining LWP (Table 3) and PSA as measured by the in situ method (Table 4). Given these experimental conditions, essentially plants that were watered vs. plants not watered, an interaction between stress treatments and genotypes (selected for their relative drought resistance) would seem likely. As previously mentioned, this interaction between genotypes and stress treatments was not detected for PSA determined by destructive acetylene reduction. These results illustrate the usefulness of in situ acetylene reduction for detecting changes in physiological processes over time due to environmental stress.

The value of the in situ method for detecting gradual changes in PSA during changes in environmental conditions is evident in Fig. 1. Since the stress treatments began at the date of first harvest, no differences between stress treatments were apparent at 58 DAP. However, large differences between stress treatments were found at 63 and 71 DAP, suggesting that in situ acetylene reduction was useful for measuring the effect of gradual water stress on N_2 fixation.

Of the N_2 fixation variables studied, PSA appeared to be affected by stress to a greater extent than NW or NN. However, this effect is a reflection of the timing of stress induction and the fact that physiological processes, such as nitrogenase activity, respond much more rapidly to stress than morphological processes, such as nodulation and leaf abscission. Also, stress

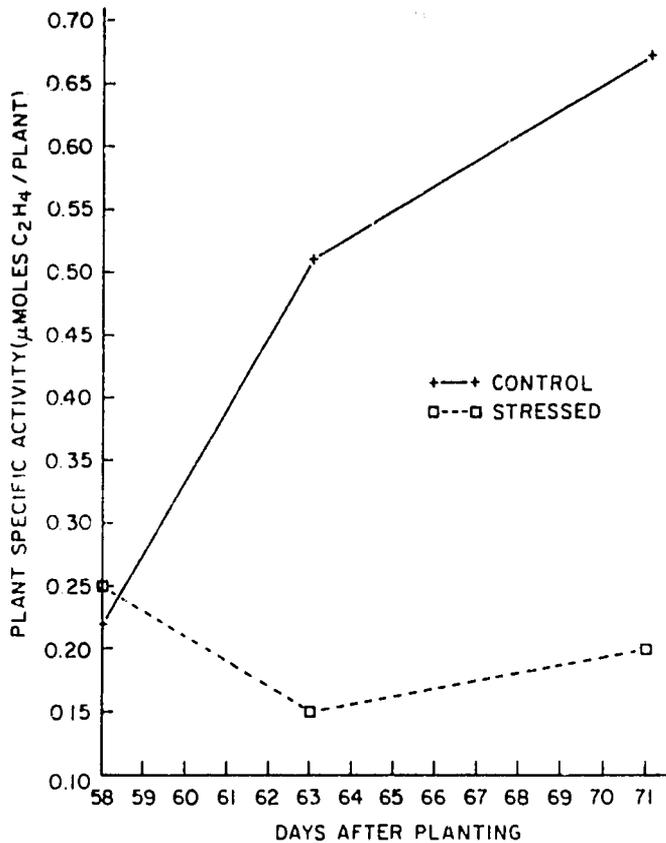


Fig. 1. Plant specific activity as measured by the in situ method, 58, 63, and 71 days after planting, Fall 1981. The interaction between stress treatment and date was significant at the 0.05 level.

may have become effective after the period of primary nodulation and nodule enlargement.

In the Spring 1982 experiment, ANOVA revealed significant interactions between stress treatment and genotype for SFW and SDW as well as RFW determined on 25 Mar., 5 Apr., and 19 Apr. 1982. Significant differences between resistant and susceptible genotypes were found for SFW and SDW, and resistant genotypes had higher values than susceptible genotypes in the stress treatment. When the genotype \times stress treatment interaction was found to be significant, differences between resistant and susceptible genotypes were distinct in the water stress treatment; hence, genotypic means for shoot fresh and dry weights of plants grown in the stress treatment are reported in Table 5.

Intraspecific variability for root exploration, a drought avoidance mechanism, is suggested by the significant differences in taproot length found between resistant and susceptible genotypes (Table 5). Water stress reduced taproot length; however, an interaction between stress treatments and genotypes was not detected. Mean taproot length for plants in the control and stress treatments was 171 and 149 mm, respectively. This difference represents a 13% reduction. Although enhanced root exploration has not been reported as a drought avoidance mechanism in cowpea, evidence for differences between resistant and susceptible genotypes for taproot length was found among the 5 genotypes used in the Spring 1982 experiment. More specific and precise techniques could be used to evaluate genotypic root exploration potential.

Interactions between sampling date and stress treatment were significant for LWP, SFW, SDW, RFW, NW, NN, and PSA, as measured by in situ acetylene reduction. These interactions

Table 5. The influence of genotype on shoot fresh and dry weight and taproot length in the stress treatment, Spring 1982.

Genotype ^a	Stress treatment		
	Shoot weight (g)		Taproot length (mm)
	Fresh	Dry	
Resistant ^b			
TVu 129	28.7	5.7	167
TVu 966	29.0	4.0	165
Susceptible			
TVu 6441	20.9	3.4	112
TVu 6565	21.7	2.9	137
Check			
California			
Blackeye No. 5	25.2	5.4	163
SE ^c	2.9	0.6	11.2

^aTVu numbers as cited in Cowpea Germplasm Catalog, No. 1, 1974, International Institute of Tropical Agriculture, Ibadan, Nigeria.

^bA contrast of resistant vs. susceptible genotypes was significant at the 0.05 level for all variables. The interaction between genotype and treatment was significant at the 0.05 level for shoot fresh and dry weight.

^cSE = Standard error of a difference between 2 means.

were expected, since stress was imposed on the first harvest date and its effects would be expected at harvest dates. In general, large differences between treatments were not noted until the final harvest date, suggesting that the onset of stress in this experiment was gradual. At 81 DAP, mean SFW was 45.6 and 21.1 g for the control and stress treatments, respectively — for a 54% reduction in SFW due to stress. Shoot dry weight at 81 DAP was 6.6 and 3.7 g in the control and stress treatments, respectively—a reduction of 44%. Mean RFW was reduced from 14.2 to 4.3 g as a result of the stress treatment for a reduction of 70% at 81 DAP, compared to 32% in the previous experiment. At 81 DAP, LWP was -9.0 and -13.5 bars for control and stress treatments, respectively. Nodule number/plant was 83 and 38 for control and stress treatments, respectively, representing a 54% reduction due to stress. Nodule fresh weight was 1.71 and 0.31 g in control and stress treatments, respectively.

The interactions between sampling date and stress treatment for NN and NW were significant (Fig. 2). Differences between stress treatments for NN and NW were not detected until 81 DAP. The decline of NN and NW indicated that nodule abscission may have occurred in stressed plants. In previous research using peas (*Pisum sativum*) and red kidney beans (*Phaseolus vulgaris* L.), Wilson (19, 20) discovered that a reduction in soil moisture levels resulted in nodule abscission. He suggested that nodules located on small, fibrous roots would be more likely to abscise than nodules on or near the taproot. Our observations indicated that taproot nodules begin senescence and abscission earlier than nodules on fibrous roots under both stress and non-stress environments (13). The greater impact of water stress on NW, as compared to NN, suggests that either larger nodules abscised first or that stressed nodules were shrinking due to a loss of water. In general, ANOVA for both experiments indicated that the effect of water stress on N₂-fixation variables was far greater than the influence of genotype.

A significant interaction between sampling date and stress treatment was found for PSA measured by the in situ method. While in situ PSA in both stress and control treatments decreased over time, the decline was greater in the stress treatment

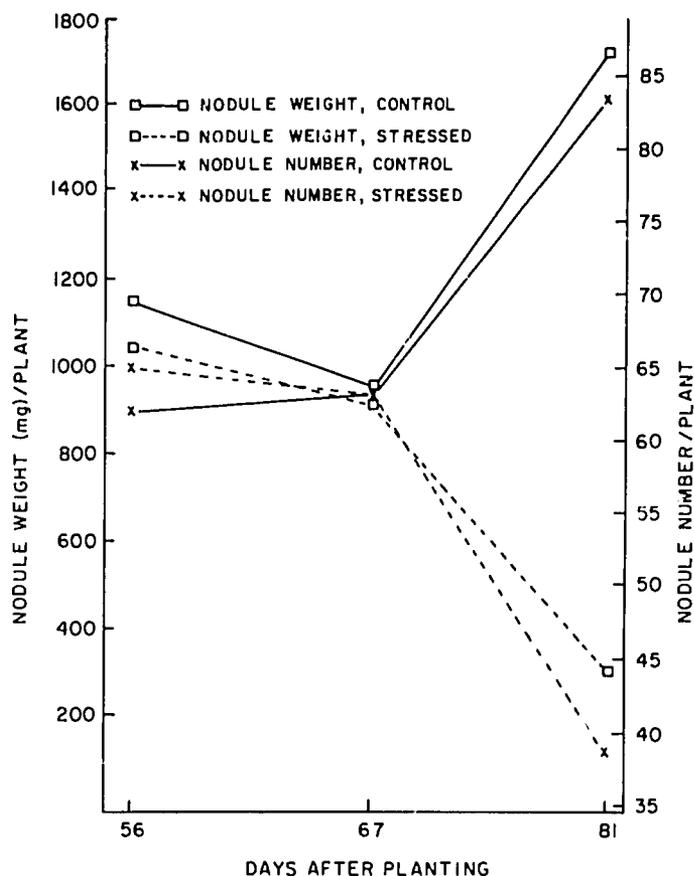


Fig. 2. Nodule weight and number, 56, 67, and 81 days after planting, Spring 1982.

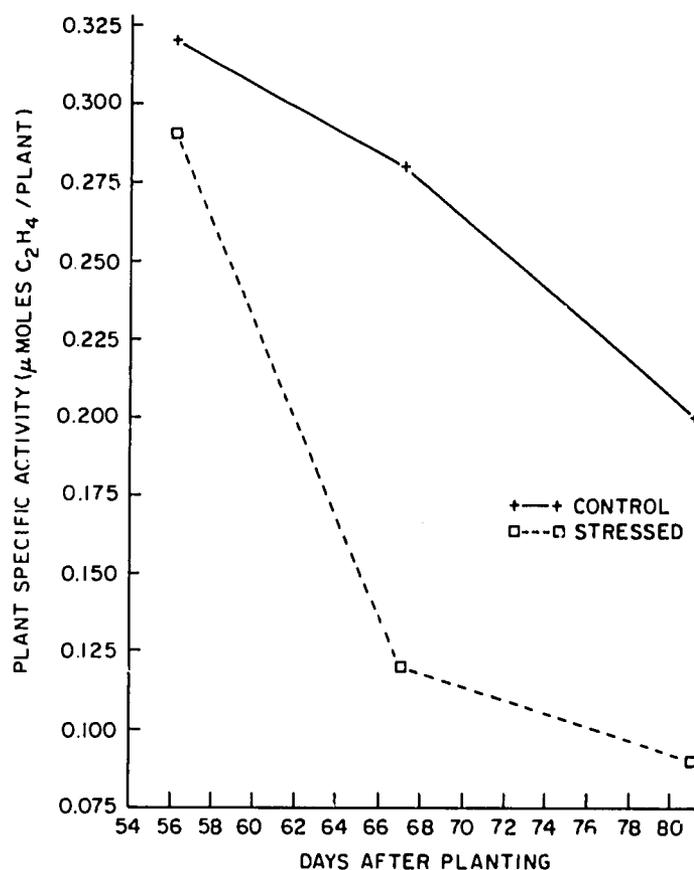


Fig. 3. Plant specific activity as measured by the in situ method, 56, 67, and 81 days after planting, Spring 1982. The interaction between stress treatment and date was significant at the 0.05 level.

than the control treatment between 56 and 67 DAP (Fig. 3). In comparing the results of in situ acetylene reduction in both experiments, in situ PSA of non-stressed plants increased over time (DAP) during the Fall 1981 experiments, while it decreased in the Spring 1982 experiment. Differences in the physiological age of the plants and in greenhouse temperatures may account for the overall declines in PSA during the sampling period in 1982. Similar reductions over time were detected by the destructive method in the experiment with repeated observations. Plant specific activity measured by the destructive method was 20.2, 8.8, and 6.9 $\mu\text{mol C}_2\text{H}_4 \cdot \text{plant}^{-1} \cdot \text{hr}^{-1}$ 56, 67, and 81 DAP, respectively. Plant specific activity from the destructive method was 16.1 and 7.9 $\mu\text{mol C}_2\text{H}_4 \cdot \text{plant}^{-1} \cdot \text{hr}^{-1}$ in the control and stress treatments, respectively. However, in situ PSA determination may have been more sensitive to stress, as illustrated by the interaction between sampling date and stress treatment (Fig. 3).

A significant interaction between genotype and stress treatment was not detected for either in situ or destructive PSA. Genotypic differences were found using both methods. Except for TVu 6565, genotypic differences (as determined by the 2 methods) generally agreed (Table 6). In using the in situ method, TVu 6565 was found to have the highest nitrogenase activity but was in the middle range of activity when the destructive method was used. These results were compared to that of a previous experiment (13) and it appeared that TVu 6565 was unusually low in PSA for the destructive method in the spring experiment. This observation is supported by the fact that TVu 6565 had the highest PSA as measured by both methods during the Fall 1981 experiment (data not shown).

Table 6. The influence of genotype on plant specific activity as measured by destructive and in situ methods, Spring 1982.

Genotype ^a	Plant specific activity	
	Destructive ^b	In situ ^c
California Blackeye No. 5	9.78 b ^w	0.18 c
TVu 129	8.89 b	0.17 c
TVu 966	18.14 a	0.22 b
TVu 6441	12.66 ab	0.23 b
TVu 6565	10.56 b	0.28 a
SE ^d	1.96	0.01

^aTVu numbers as cited in Cowpea Germplasm Catalog, No. 1, 1974, International Institute of Tropical Agriculture, Ibadan, Nigeria.

^b $\mu\text{mol C}_2\text{H}_4 \cdot \text{plant}^{-1} \cdot \text{hr}^{-1}$.

^c $\mu\text{mol C}_2\text{H}_4 \cdot \text{plant}^{-1}$.

^wMean separation within columns by Duncan's multiple range test, 5% level.

^dSE = Standard error of a difference between 22 means.

Correlation coefficients between in situ PSA, destructive PSA, NW, and NN were calculated (Table 7). The correlation coefficients between destructive PSA and in situ PSA sampled 10 and 30 min after injection with acetylene were 0.67 and 0.70, respectively. Destructive PSA and in situ PSA were more highly correlated with each other than destructive PSA and NW or NN (0.62 and 0.42, respectively), or NW and NN (0.64). Nodule weight and number, however, were more highly correlated with destructive PSA than PSA measured by the in situ method. Nevertheless, measurement of PSA by in situ acetylene reduction may be a relatively simple, rapid and nondestructive means

Table 7. Correlation coefficients between plant specific activity measured by the in situ method, sampled 10 and 30 min after injection, and the destructive method, nodule weight, and nodule number on 25 Mar., 5 Apr., and 19 Apr. 1982.

	Plant specific activity		Nodule weight	Nodule number
	In situ (30 min)	Destructive		
PSA in situ				
10 min	0.93 [†]	0.67	0.36	0.36
30 min	---	0.70	0.37	0.34
PSA destructive		---	0.62	0.45
Nodule weight			---	0.64

[†]All correlation coefficients were significant at the 0.05 level.

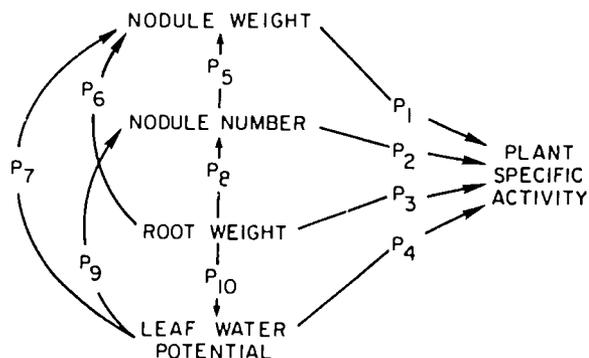


Fig. 4. Path analysis diagram on N₂ fixation variables studied.

for estimating N₂ fixation. Although the in situ acetylene reduction procedure used was less sophisticated and simpler than other published techniques (6), it was found to be quite useful for detecting large differences in PSA due to the stress treatments in greenhouse-grown plants.

In order to ascertain which N₂-fixation variables were most important for determining N₂ fixation during water stress, path analysis was used (5). The path analysis diagram in Fig. 4 depicts the development of N₂ fixation in legumes. Path analysis permits the partitioning of a total correlation coefficient into several components including direct effect, numerous indirect effects, and spurious effects (5). Path coefficients were determined by path analysis for 3 groups of data, namely plants assigned to each of the 2 treatments and all data adjusted for the effects of treatments. Adjustment for the effects of treatments was achieved by conducting a regression-type analysis with treatments and blocks as independent variables. Residuals were used in the path analysis, after the effects of treatments and blocks had been removed.

Results of the Fall 1981 experiment showed that while the total correlation between NW and destructive PSA was high (0.75 and 0.90), when data were adjusted for treatments and for plants in the control treatment, respectively, total correlation was low in the stress treatment (0.16) (Table 8). In fact, the direct effect of NW on PSA was negligible for plants in the stress treatment, and indirect effects of NN were more important than NW in stressed plants. The direct effect of NN on PSA was 0.48 in the stress treatment, but not as important in non-stressed plants. Nodule weight had the largest (0.60) indirect effect on PSA via NN in nonstressed plants. Root fresh weight was not found to be important in determining PSA in any of the 3 groups of data. However, LWP was found to be important when data were adjusted for treatments and for plants grown in

Table 8. Coefficients from path analysis of the relationships among N₂ fixation variables in stressed and nonstressed plants, Fall 1981.

Variables	Correlation and path coefficients		
	Adjusted for treatments	Stressed	Nonstressed
Nodule weight vs. plant specific activity			
Direct effect	0.56	-0.02	0.83
Indirect effects via:			
Nodule number	0.13	0.24	0.02
Other variables	0.07	-0.06	0.05
Correlation	0.75	0.16	0.90
Nodule number vs. plant specific activity			
Direct effect	0.19	0.48	0.03
Indirect effects via:			
Nodule weight	0.39	-0.01	0.60
Leaf water potential	0.01	-0.12	0.03
Other variables	0.04	0.00	0.08
Correlation	0.62	0.16	0.90
Root fresh weight vs. plant specific activity			
Direct effect	0.07	0.02	0.06
Indirect effects via:			
Nodule number	0.04	0.10	0.01
Nodule weight	0.13	-0.01	0.20
Nodule number-nodule weight	0.08	0.00	0.17
Other variables	-0.03	-0.02	-0.10
Correlation	0.30	0.09	0.34
Leaf water potential vs. plant specific activity			
Direct effect	0.28	0.48	0.09
Indirect effects via:			
Nodule weight	0.05	0.00	0.10
Nodule number	0.01	-0.12	0.01
Nodule number-nodule weight	0.01	0.00	0.23
Other variables	-0.03	-0.01	-0.10
Correlation	0.33	0.36	0.33

the stress treatments. Correlations between LWP and PSA were 0.29 and 0.48, respectively, for data adjusted for treatments and plants grown in the stress treatment. Factors other than LWP were more important for plants grown in the control treatment.

In the Spring 1982 experiment, NW was found to have a large direct effect on PSA in all 3 groups of data (Table 9). Nodule number was not found to influence PSA greatly, except for plants grown in the control treatment, where the direct effect of NN on PSA was almost as great as the indirect effect due to NW. Path analysis revealed a crucial relationship between LWP and PSA in all 3 groups of data in the 1982 experiment. In stressed plants, increased LWP was closely associated with higher PSA. Evidence has been presented to suggest that intraspecific variability for drought avoidance and LWP exists. Therefore, drought resistant genotypes that may maintain a high plant water status should have higher N₂ fixation potential during water stress than susceptible genotypes. The development of drought-resistant cultivars should indirectly improve N₂ fixation potential.

Path analysis also revealed that stressed plants with increased nodule number had increased PSA in the fall experiment. However, in the spring experiment, NW was the most important N₂

Table 9. Coefficients from path analysis of the relationships among N₂ fixation variables in stressed and nonstressed plants, Spring 1982.

Variables	Correlation and path coefficients		
	Adjusted for treatments	Stressed	Nonstressed
Nodule weight vs. plant specific activity			
Direct effect	0.62	0.71	0.58
Indirect effects via:			
Nodule number	0.07	-0.03	0.13
Root weight	-0.09	-0.05	-0.11
Other variables	0.08	0.09	0.07
Correlation	0.67	0.72	0.67
Nodule number vs. plant specific activity			
Direct effect	0.14	-0.04	0.27
Indirect effects via:			
Nodule weight	0.34	0.46	0.28
Other variables	0.05	0.07	0.04
Correlation	0.53	0.49	0.58
Root fresh weight vs. plant specific activity			
Direct effect	-0.25	-0.18	-0.28
Indirect effects via:			
Nodule weight	0.23	0.18	0.22
Nodule number	0.03	0.00	0.09
Nodule number-nodule weight	0.08	0.03	0.10
Other variables	0.03	-0.02	0.08
Correlation	0.12	0.00	0.21
Leaf water potential vs. plant specific activity			
Direct effect	0.29	0.29	0.24
Indirect effects via:			
Nodule weight	0.12	0.15	0.12
Other variables	0.07	0.07	0.08
Correlation	0.47	0.51	0.43

fixation variable affecting PSA in both stressed and nonstressed plants. Differences between the 2 experiments could be explained by the number of harvest dates involved. In the fall experiment, measurements were made once after plants had been stressed for some time. In the spring experiment, measurements were made over a wider range of time during a period of gradual water stress imposition. In addition, it is difficult to impose precisely a water stress at a particular stage of nodule development. Although stress treatments were applied at nearly the same number of DAP in both experiments, it is possible that plants in the fall experiment were stressed at the time of nodulation, while plants in the spring experiment were at a more advanced physiological age. Furthermore, the extent of stress in both experiments apparently was different, with plants in the fall experiment experiencing stress at a less advanced physiological age than those in the spring experiment. The critical issue here is the recognition of different stages of nodule development and how N₂ fixation can be affected at each stage. It is probably incorrect to state that stress at a particular stage of growth is more or less detrimental to N₂ fixation than stress at another stage. Stages of N₂ fixation development in a legume are interrelated and interdependent, and the importance of stress at any stage of development cannot be underestimated.

The results of this study indicate that water stress has a strong and negative impact on N₂ fixation. In both experiments, the

effect of water stress on N₂ fixation variables was far greater than the influence of genotype, when genotypes were selected for relative drought resistance. Maintenance of LWP via drought avoidance should increase N₂ fixation potential during water stress. Therefore, breeding for drought avoidance in conjunction with N₂ fixation may be more beneficial than breeding for N₂ fixation potential. Intraspecific variability in cowpea for several drought avoidance mechanisms is suggested, thus offering the potential to select and breed for enhanced drought resistance in cowpea. Within the group of cowpea genotypes identified as drought resistant (14), it should be possible to identify additional drought-avoidance mechanisms.

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